Supporting Information

Figure S1. Clinal *foxo* candidate SNPs. (A) Allele frequencies of clinal *foxo* SNPs in Florida (red), Pennsylvania (green) and Maine (blue), identified by Fabian et al. (2012) and conditioned to raise in frequency from Florida to Maine. The two strongly clinal *foxo* SNPs studied here are marked with star symbols. Note that the SNP in-between the two focal SNPs is much less strongly clinal, with a much higher frequency in Florida than the 2 candidate SNPs. The x-axis shows the genomic position of the SNPs on chromosome *3R* in million base pairs (Mbp). The plot underneath the x-axis shows the gene model for *foxo*. (B) Linkage disequilibrium (LD; as measured by pairwise r^2) among all polymorphic *foxo* SNPs (minor allele frequency ≥ 0.1) in the DGRP lines used to set up experimental populations (see Materials and Methods section). The two focal SNPs are in perfect LD in the experimental populations (r^2 =1), but there is no significant LD among other, non-focal sites. Nonetheless, we cannot rule out with certainty that other SNPs are in LD with our two focal SNPs; a cautious interpretation would thus be to view our focal SNPs as representing "tag SNPs". Also see Fig. S3; also see analyses in Betancourt et al. (2018).

Figure S2. PEST motif prediction for FOXO. The T/G polymorphism in *foxo* at position *3R*: 9894559, is predicted to be located in the PEST region of the FOXO protein (analysis of *foxo* sequence using ExPASy [Artimo et al., 2012]); PEST motifs serve as protein degradation signals (Artimo et al., 2012). The potential PEST motif (RPENFVEPTDELDSTK) between amino acid positions 49 and 64 (shown in green) encompasses the *foxo* SNP at position 51 (E = glutamic acid).

Figure S3. Experimental design for reconstituted outbred *foxo* populations. We isolated the 2-SNP *foxo* variant by reconstituting outbred populations, fixed for either the low- or high-latitude allele, from lines of the *Drosophila* Genetic Reference Panel (DGRP). Each *foxo* allele was represented by two independent sets of distinct DGRP lines, with two replicate cages per set. See Materials and Methods section for details; also see Fig. S1B; also see analyses in Betancourt et al. (2018).

Figure S4. Coordinates of landmarks used to estimate wing area. We calculated the total wing area encompassed by 12 landmarks (in yellow) by splitting the polygon up into triangles (shown in different colors) and by summing across the areas defined by these triangles. See Materials and Methods section for details.

Figure S5. Effects of the *foxo* variant on total wing area. Effects of the clinal *foxo* variant on wing area (mm^2) in females and males. (A) Dietary reaction norms at 18°C. (B) Dietary reaction norms at 25°C. (C) Thermal reaction norms on sucrose diet. (D) Thermal reaction norms on molasses diet. Shown are means and standard errors. Red lines: low-latitude (LL) allele, blue lines: high-latitude (HL) allele. See Results section for details.

Figure S6. Effects of the *foxo* variant on thorax length. Effects of the clinal *foxo* variant on thorax length (mm) in females and males. (A) Dietary reaction norms at 18°C. (B) Dietary reaction norms at 25°C. (C) Thermal reaction norms on sucrose diet. (D) Thermal reaction norms on molasses diet. Shown are means and standard

errors. Red lines: low-latitude (LL) allele, blue lines: high-latitude (HL) allele. See Results section for details.

Figure S7. Effects of the *foxo* variant on male survival upon starvation. Effects of the clinal *foxo* polymorphism on the duration of survival (in hrs) upon starvation in males. (A) Dietary reaction norms at 18°C. (B) Dietary reaction norms at 25°C. (C) Thermal reaction norms on sucrose diet. (D) Thermal reaction norms on molasses diet. Shown are means and standard errors. Red lines: low-latitude (LL) allele, blue lines: high-latitude (HL) allele. See Results section for details.

Figure S8. Effects of the *foxo* variant on relative abundance of insulin-like receptor (InR) transcription levels. (A) Low-latitude (LL) allele has higher level of InR transcription than the high-latitude (HL) allele. (B) Carbohydrate-rich molasses diet resulted in more InR transcripts than the sucrose diet. Shown are means and standard errors. See Results section for details.

Figure S1

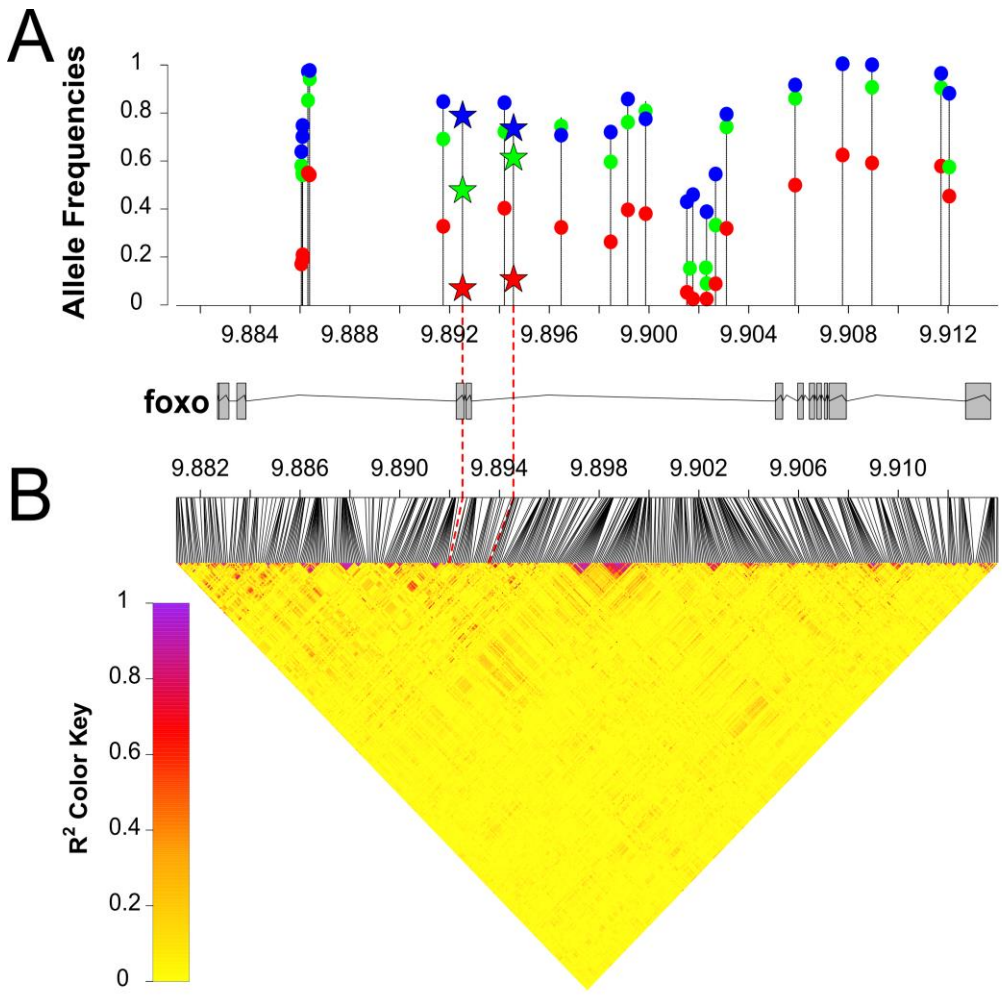
A
Allele Frequencies
1
0.8
0.6
0.4
0.2
0
9.884
9.888
9.892
9.896
9.900
9.904
9.908
9.912
foxo
B
9.882
9.886
9.890
9.894
9.898
9.902
9.906
9.910
R2 Color Key
1
0.8
0.6
0.4
0.2
0

Figure S2

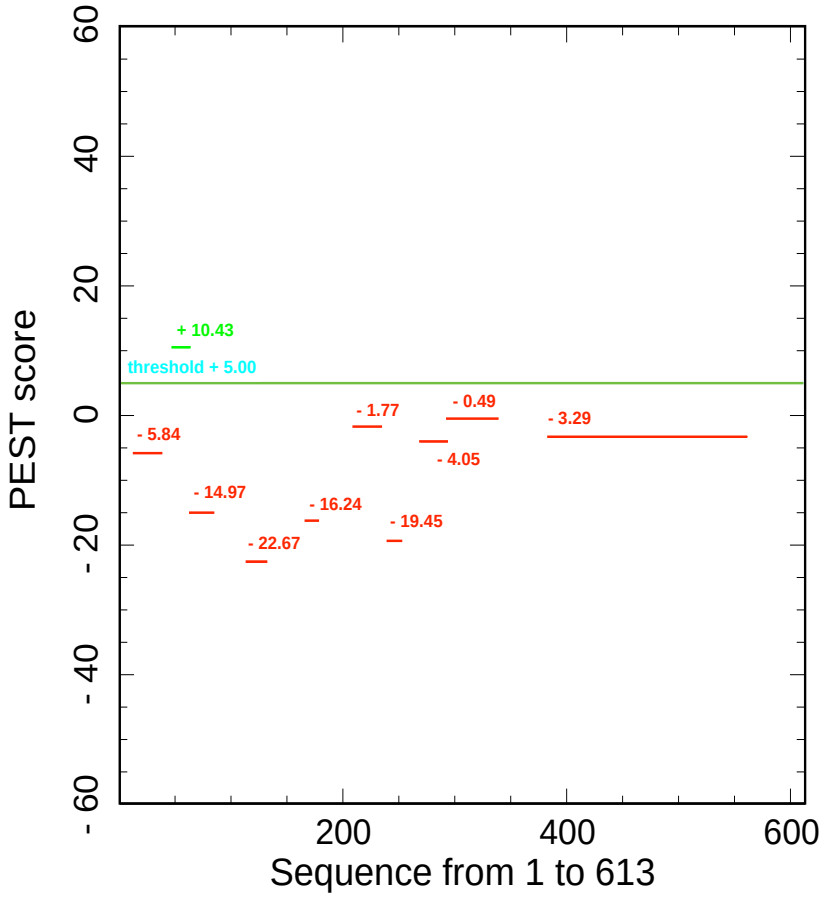
PEST score
60
40
20
0
- 20
- 40
- 60
200
400
600
Sequence from 1 to 613
+ 10.43
threshold + 5.00
- 5.84
- 14.97
- 22.67
- 16.24
- 19.45
- 1.77
- 4.05
- 0.49
- 3.29

Figure S3

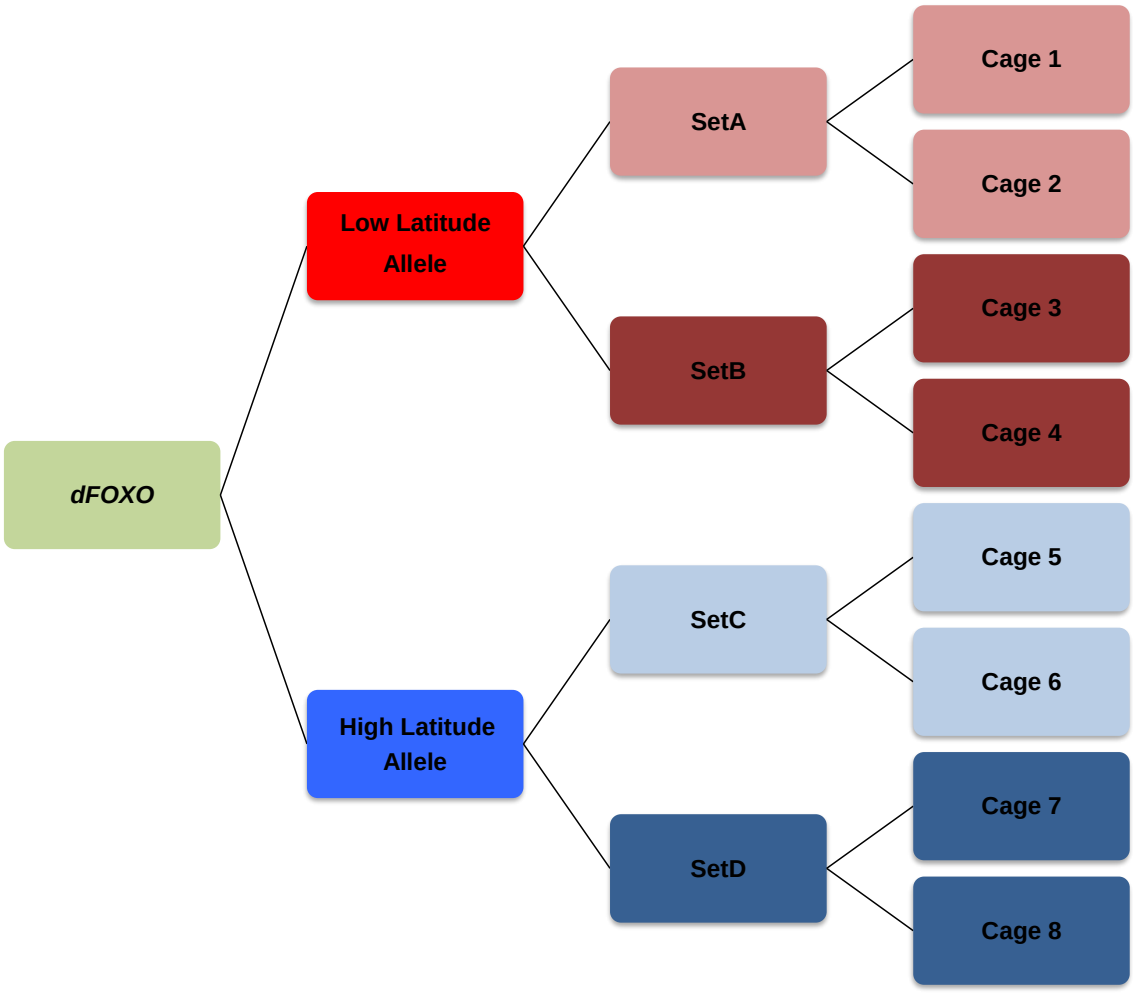
dFOXO
Low Latitude Allele
High Latitude Allele
SetA
SetB
SetC
SetD
Cage 1
Cage 2
Cage 3
Cage 4
Cage 5
Cage 6
Cage 7
Cage 8

Figure S4

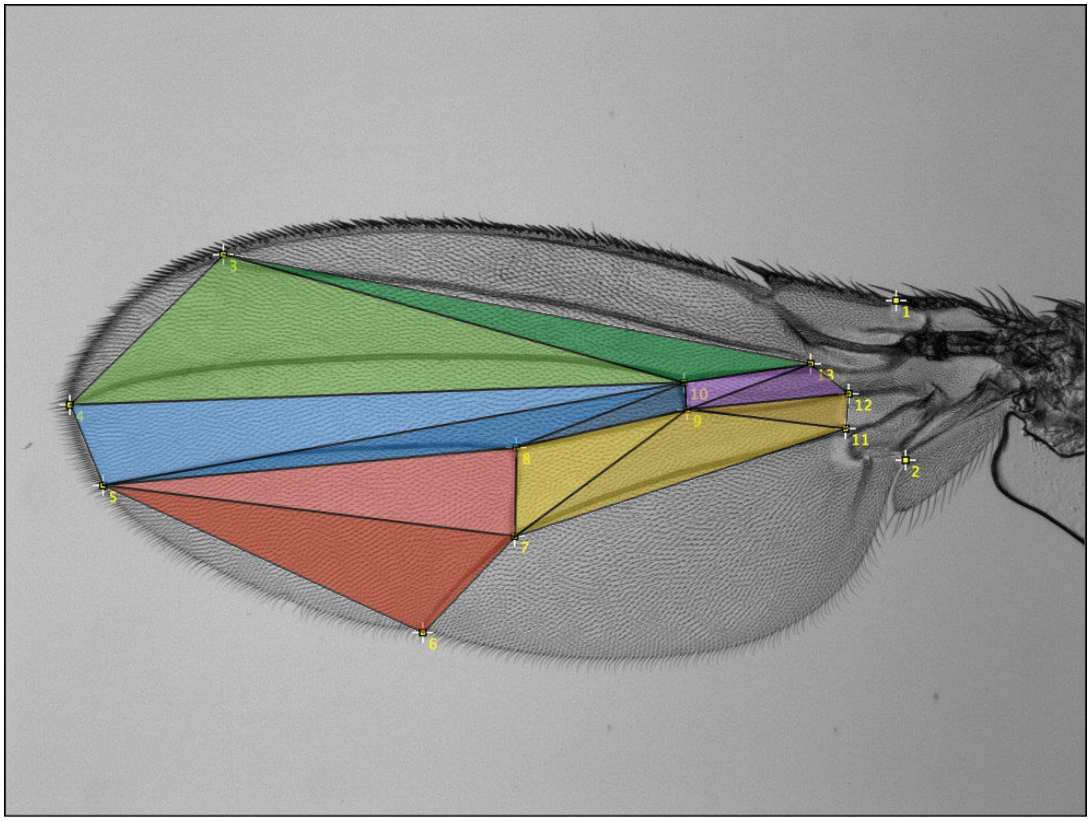

Figure S5

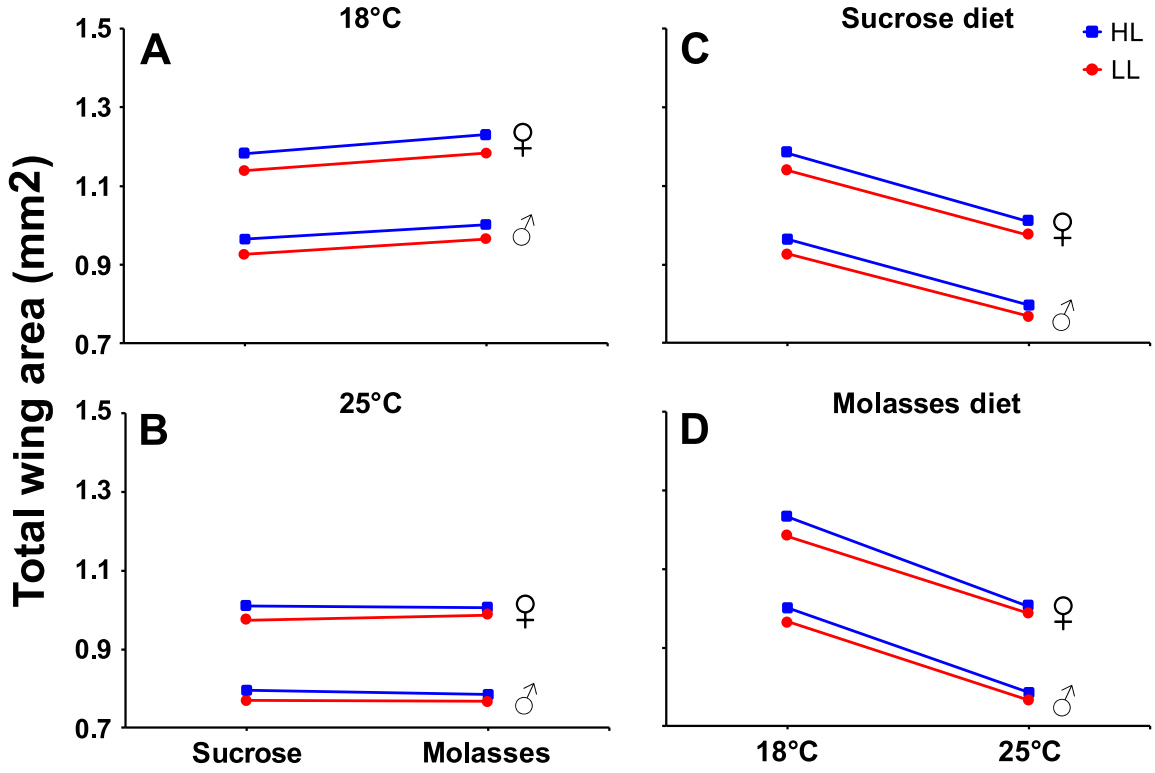
Total wing area (mm2)
1.5
1.3
1.1
0.9
0.7
A
18°C
B
25°C
C
Sucrose diet
D
Molasses diet
HL
LL
♀
♂
Sucrose
Molasses
18°C
25°C

Figure S6

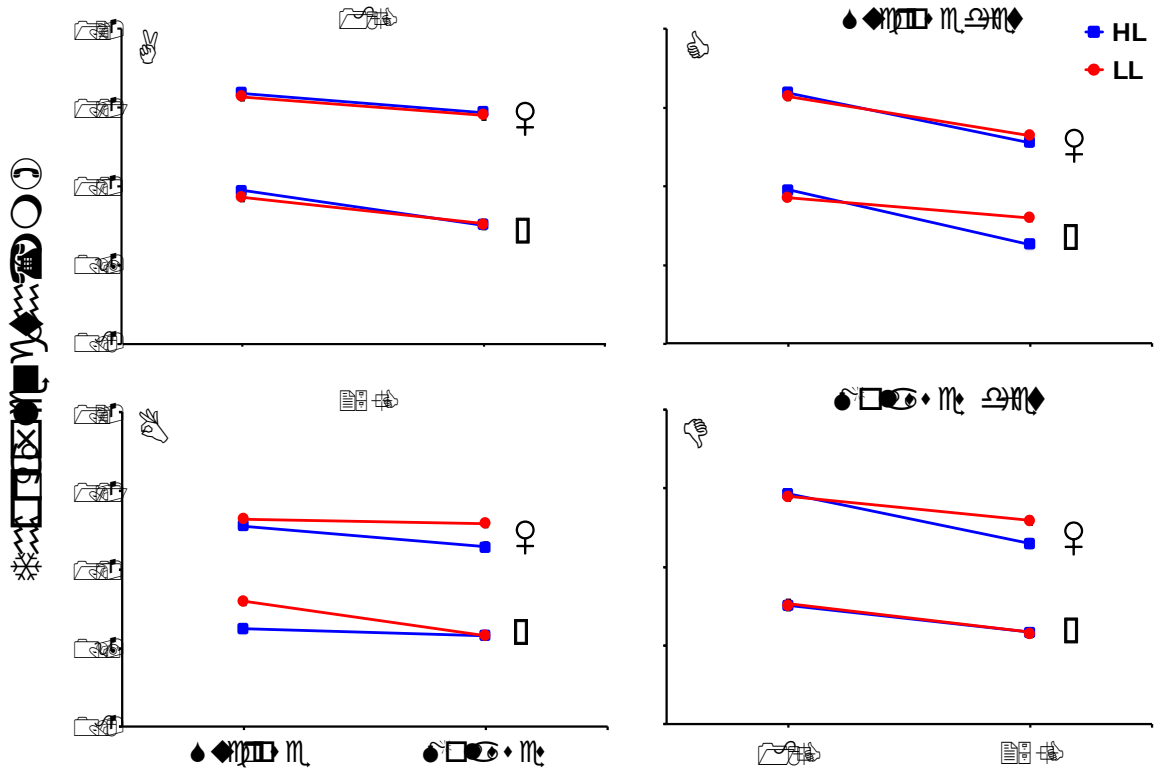

Figure S7

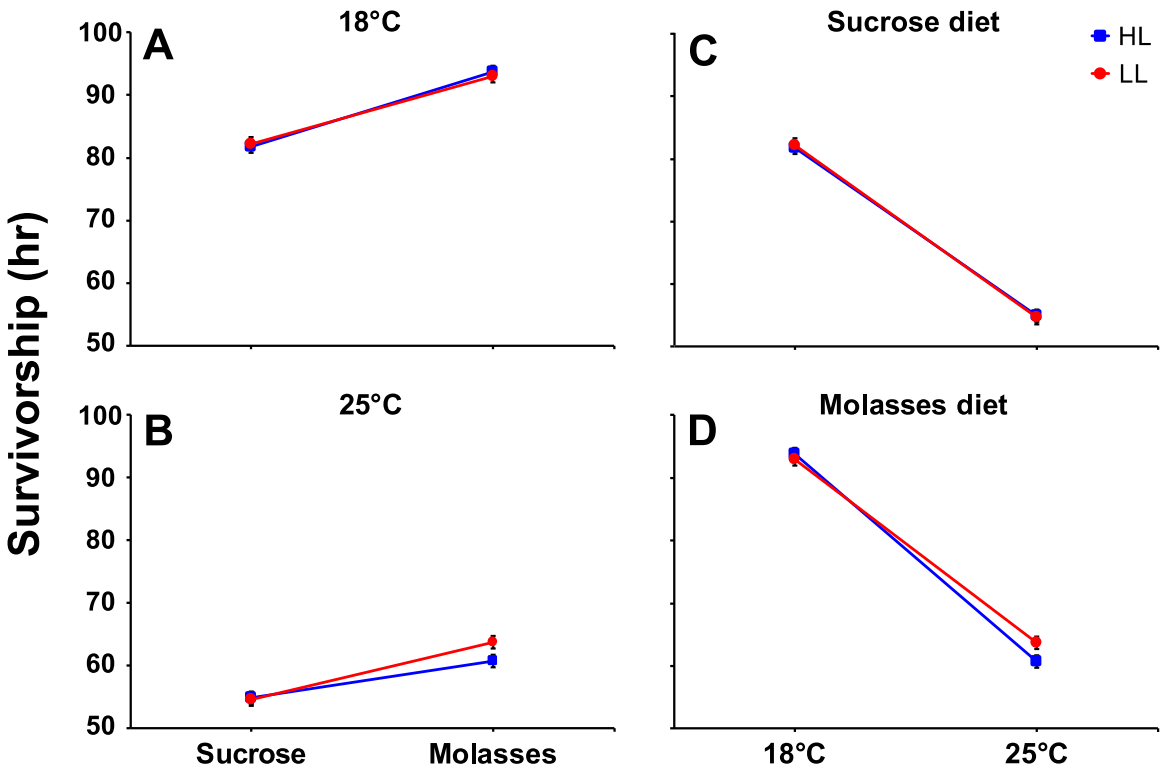
Survivorship (hr)
A
18°C
B
25°C
C
Sucrose diet
D
Molasses diet
HL
LL
100
90
80
70
60
50
Sucrose
Molasses
18°C
25°C

Figure S8

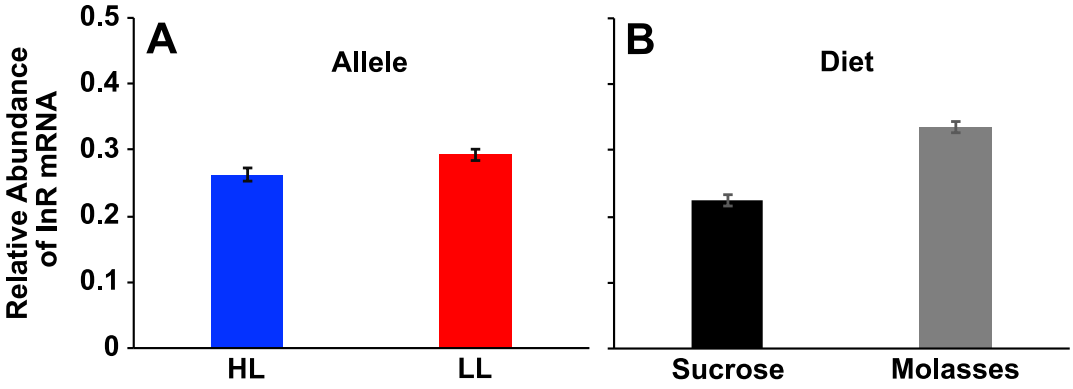
Relative Abundance of InR mRNA
0.5
0.4
0.3
0.2
0.1
0
A
Allele
HL
LL
B
Diet
Sucrose
Molasses

Table S1. Details of design of reconstituted outbred population cages. HL: high-latitude *foxo* allele; LL: low-latitude *foxo* allele. See Materials and Methods section for details.

Allele	Position	Set	Cage number	DGRP lines
LL	3R:9892517 + 9894559 (GG)	A	1	26, 57, 73, 75, 91, 101, 105, 161, 176, 280, 313, 318, 367, 371, 375, 377, 378, 379
LL	3R:9892517 + 9894559 (GG)	A	2	26, 57, 73, 75, 91, 101, 105, 161, 176, 280, 313, 318, 367, 371, 375, 377, 378, 379
LL	3R:9892517 + 9894559 (GG)	B	3	208, 373, 406, 426, 440, 491, 492, 508, 513, 535, 639, 646, 757, 761, 796, 805, 812, 852
LL	3R:9892517 + 9894559 (GG)	B	4	208, 373, 406, 426, 440, 491, 492, 508, 513, 535, 639, 646, 757, 761, 796, 805, 812, 852
HL	3R:9892517 + 9894559 (AT)	C	5	40, 41, 42, 69, 83, 109, 142, 153, 158, 177, 195, 229, 233, 365, 370, 380, 391, 405
HL	3R:9892517 + 9894559 (AT)	C	6	40, 41, 42, 69, 83, 109, 142, 153, 158, 177, 195, 229, 233, 365, 370, 380, 391, 405
HL	3R:9892517 + 9894559 (AT)	D	7	45, 332, 338, 443, 517, 531, 595, 703, 705, 707, 774, 790, 804, 820, 837, 855, 879, 890
HL	3R:9892517 + 9894559 (AT)	D	8	45, 332, 338, 443, 517, 531, 595, 703, 705, 707, 774, 790, 804, 820, 837, 855, 879, 890

Table S2. Nutritional value and composition of sucrose and molasses diets. Table S2a: nutritional values of fly food ingredients per 100 g; Table S2b: recipe for sucrose and molasses diets; Table S2c: comparison of nutritional values of sucrose and molasses diets. See Materials and Methods section for details. The sucrose diet is the standard medium used in our laboratory in Lausanne; the recipe for the molasses diet follows that recipe of the Bloomington *Drosophila* Stock Center (BDSC) but uses different products for the food ingredients. The principal (but not exclusive) differences between the two diets are their carbohydrate source (sucrose vs. molasses) and their protein:carbohydrate (P:C) ratios.

S2a. Nutritional values of ingredients in 100g of fly food

	Yeast	Cornmeal	Sucrose	Molasses
Energy (kcal)	310	345	400	290
Protein (g)	45	8	0	0
Total carbohydrates (g)	15	74	100	75

S2b. Food recipes for sucrose and molasses diets

	Sucrose	Molasses
Cornmeal (g/L) (*Polenta, Migros*)	50	61.3
Yeast (g/L) (*Actilife, Migros*)	50	12.4
Sugar (g/L) (*Cristal, Migros*)	50	0
Molasses (g/L) (*Zuckerrohrmelasse, EM Schweiz*)	0	109.6
Agar (g/L) (*Drosophila Agar Type II, Genesee*)	7	6
Nipagin 10% (ml/L) (*Sigma Aldrich*)	10	14.3
Propionic acid (ml/L) (*Sigma Aldrich*)	6	6

S2c. Nutritional values of sucrose and molasses diets

	Sucrose	Molasses
Energy (kcal)	527.50	567.77
Protein (g/L)	26.50	10.48
Total carbohydrate (g/L)	94.50	129.42
P:C ratio	~ 1:3.6 (≈0.28)	~1:12.3 (≈0.08)

Table S3. Summary of effect size estimates (Cohen's *d*) for viability, femur length, wing area, thorax length, starvation resistance, and fat (TAG) content. White and grey cells show results for females and males, respectively. *d* = 0.01, very small; *d* = 0.20, small; *d* = 0.50, medium; *d* = 0.80, large; *d* = 1.20, very large.

Factor	18°C Sucrose diet	18°C Molasses diet	25°C Sucrose diet	25°C Molasses diet
Viability	0.49	0.50	0.54	0.89
Femur Length	0.09	0.17	0.49	0.00
	0.25	0.05	0.14	0.20
Wing Area	0.59	0.67	0.66	0.35
	0.68	0.62	0.72	0.48
Thorax Length	0.13	0.08	0.20	0.81
	0.26	0.07	1.15	0.00
Starvation Resistance	0.11	0.34	0.24	0.51
	0.03	0.04	0.03	0.26
TAG content (Fed)	0.19	0.70	0.25	0.72
TAG content (Starved)	0.72	0.24	0.04	0.04

Table S4. Summary of ANOVA results for wing area, thorax length, and male starvation resistance (also cf. Table S5). White and grey cells show the results for females and males, respectively; data for starvation resistance are for males only. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. See Results section for details.

Factor in ANOVA	Total Wing Area	Thorax Length	Starvation Resistance
Allele	$F_{1,32}=105.39^{***}$	$F_{1,32}=4.33^{*}$	$F_{1,32}=0.70$
	$F_{1,32}=103.87^{***}$	$F_{1,32}=3.78$	
Temperature	$F_{1,912}=2852.52^{***}$	$F_{1,422}=216.46^{***}$	$F_{1,1553}=1711.77^{***}$
	$F_{1,918}=3962.67^{***}$	$F_{1,381}=145.46^{***}$	
Diet	$F_{1,912}=48.36^{***}$	$F_{1,422}=31.90^{***}$	$F_{1,1553}=176.44^{***}$
	$F_{1,918}=28.15^{***}$	$F_{1,381}=88.62^{***}$	
Allele x Temperature	$F_{1,912}=7.15^{**}$	$F_{1,422}=10.66^{**}$	$F_{1,1553}=0.58$
	$F_{1,918}=5.89^{*}$	$F_{1,381}=8.72^{**}$	
Temperature x Diet	$F_{1,912}=35.96^{***}$	$F_{1,422}=1.67$	$F_{1,1553}=7.51^{**}$
	$F_{1,918}=56.66^{***}$	$F_{1,381}=3.48$	
Allele x Diet	$F_{1,912}=0.73$	$F_{1,422}=2.44$	$F_{1,1553}=0.58^{***}$
	$F_{1,918}=1.08$	$F_{1,381}=2.46$	
Allele x Temperature x Diet	$F_{1,912}=1.79$	$F_{1,422}=1.89$	$F_{1,1553}=2.48$
	$F_{1,918}=0.22$	$F_{1,381}=11.19^{***}$	
Set (Allele)	$F_{2,32}=53.59^{***}$	$F_{2,32}=8.05^{***}$	$F_{2,32}=1.01$
	$F_{2,32}=30.53^{***}$	$F_{2,32}=7.56^{***}$	
Cage (Set, Allele)	$F_{4,32}=64.45^{***}$	$F_{4,32}=3.41^{**}$	$F_{4,32}=12.78^{***}$
	$F_{4,32}=29.58^{***}$	$F_{4,32}=0.73$	

Table S5. Summary of REML variance component estimates for starvation resistance. White and grey cells show results for females and males, respectively.

Random Effect	Variance Ratio	Variance Component	Std Error	95% Lower	95% Upper	Wald *p*-Value	Percentage of Total
Vial(Cage,Set,Allele)	0.00	-0.19	2.96	-6.00	5.62	0.95	0.00
	0.00	0.13	1.29	-2.39	2.65	0.92	0.07
Residual		474.90	17.08	443.13	510.23		100.00
		199.07	7.14	185.78	213.85		99.93
Total		474.90	17.08	443.13	510.23		100.00
		199.21	7.08	186.03	213.85		100.00

Table S6. Summary of ANOVA results for relative abundance of *insulin-like receptor* (*InR*) transcript levels. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

Factor in ANOVA	Relative Abundance of *InR*
Allele	$F_{1,80}=4.54^{*}$
Temperature	$F_{1,80}=0.90$
Diet	$F_{1,80}=75.99^{***}$
Allele x Temperature	$F_{1,80}=0.05$
Temperature x Diet	$F_{1,80}=0.05$
Allele x Diet	$F_{1,80}=0.41$
Allele x Temperature x Diet	$F_{1,80}=0.08$
Set (Allele)	$F_{2,80}=6.53^{**}$
Cage (Set, Allele)	$F_{4,80}=5.73^{***}$